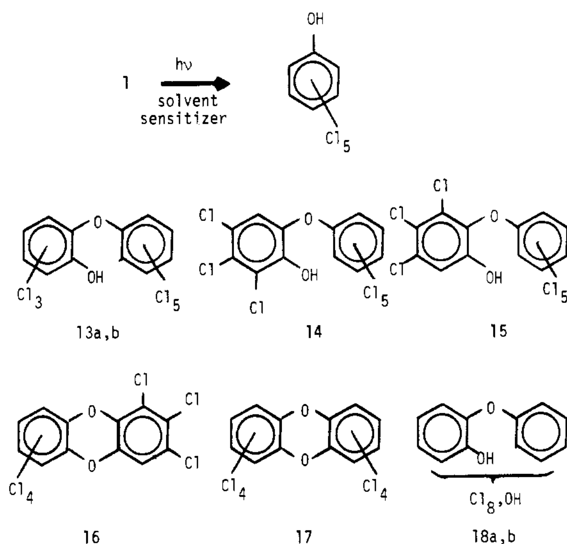
Scheme II



quantum yield for cyclization upon concentration of predioxin (1) was studied. Reactions were carried out in dibutyl ether at 300 nm, and the results are presented in Table III. Since the intermolecular process is a bimolecular process competing with unimolecular processes, one should observe an increase in cyclization with increasing concentration if this mode is operative. Instead, we see the very low amounts of cyclic product maintained, and at least qualitatively, the intramolecular process emerges as the preferred alternative.

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Enzymatic Degradation of α- and β-Cyclodextrins by *Bacteroides* of the Human Colon

Robert N. Antenucci* and James K. Palmer

Thirty *Bacteroides* strains from the human colon were tested for the ability to degrade cyclodextrins (CDs) in vitro. Twenty-four strains were able to degrade CDs. Cyclodextrinase (CDase) in two of these strains, *Bacteroides ovatus* 3524 and *Bacteroides distasonis* C18-7, has been studied. Organisms were grown on a minimal medium containing CD (0.5%), and CDase activity was assayed by measuring the increase in reducing sugar (as glucose) when CDs were incubated at 37 °C for 4 h with crude enzyme preparations. CDase activity was predominantly cell bound and induced in both organisms by growth on CDs. The products of CD hydrolysis by the crude enzyme preparations from the two strains were sharply different. *B. ovatus* 3524 CDase catalyzed production of glucose only, while the *B. distasonis* C18-7 catalyzed production of a series of maltooligomers. CDase was stable and active under expected conditions of the colon environment (pH 7.0; 37 °C).

Cyclodextrins (CDs) are cyclic oligosacchrides composed of six or more α-1,4-linked glucose units (Radley, 1968). CDs readily form inclusion complexes with various chemicals, often significantly increasing the stability and/or water solubility of the complexed compounds (Saenger, 1981). This complex formation is the basis for recent publications and patents that propose the use of CDs as nutritionally inert stabilizers in various food and pharmaceutical products. For example, CDs stabilize anthocyanin pigments (Yamada et al., 1980), increase water solubility of vitamins A, D, E, and K (Pitha et al., 1981), and stabilize food flavors, unsaturated fatty acids, and vitamin A, as well as a variety of foods including rice, cheese, and noodles (Szejtli, 1981).

In contrast to the considerable information concerning the use and role of CDs in stabilizing various food and food ingredients, the fate of ingested CDs or CD complexes is not clear. CDs are only slowly hydrolyzed by salivary or intestinal amylases (French and McIntire, 1950). This fact has been taken as evidence that CDs are metabolically inert. However, CDs could be fermented by colon anaerobes to yield products (e.g., fatty acids) that are known to have nutritive value.

CD metabolism in rats was investigated by Anderson et al. (1963) through oral administration of [^{14}C]-β-CD to rats

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and measurement of ^{14}C in exhaled CO_2. About 55% of the administered ^{14}C was exhaled between the fourth and tenth hour after administration. In a similar study by Gerloczy et al. (1981), the ^{14}C in exhaled CO_2 was measured after feeding of labeled CD or labeled glucose. About 60% of the administered ^{14}C was recovered in both cases. The ^{14}C from glucose appeared in the respired air within the first 2 h after feeding, as compared to between the fourth and eighth hour for CD. These results indicate that CDs are metabolized in the rat. When radioactivity was measured in the blood after oral administration of [^{14}C]-β-CD to rats, the radioactivity of the blood was highest between 6 and 8 h after administration (Szejtli et al., 1980). Szejtli et al. (1980) suggested that the absorption of CD does not occur from the small intestine but from the colon where CDs are presumably degraded. In vitro studies showed that mixed cultures of colon organisms from rats and rabbits were able to degrade CDs (Szabo et al., 1981).

Research concerning human metabolism of other complex carbohydrates provides evidence that certain colon organisms may be involved in the degradation and/or metabolism of various complex food carbohydrates (Salyers et al., 1977).

This report describes the in vitro degradation of CDs by human colon anaerobes, including information on properties of the enzymes catalyzing the degradation and on the products of CD hydrolysis. The results suggest that CDs are not metabolically inert in humans.

MATERIALS AND METHODS

Bacterial Strains. Previously isolated and identified human colon strains of *Bacteroides* used in this study were obtained from the Anaerobe Laboratory, Virginia Polytechnic Institute and State University. Strains were chosen based upon their ability to ferment a range of soluble polysaccharides with structures typical of those in food polysaccharides, as determined by Salyers et al. (1977). All strains had been classified into species according to DNA homology data (Johnson, 1980).

Cyclodextrins. α- and β-CDs used in the screening studies and enzymatic assays were a gift from Dr. Derek Ball, U.S. Army Natick Laboratories, Natick, MA. β-CD used in growth and enzymatic assays was from Sigma Chemical Co. (St. Louis, MO.). All CDs yielded a single peak in analyses by high-performance liquid chromatography (HPLC).

Screening for Fermentation of CDs by *Bacteroides*. The CD hydrolyzing ability of *Bacteroides* strains was tested by using the replicator method of Wilkins and Walker (1975). Microtiter plates containing α- and β-CD test media were inoculated with 24-h cultures grown in chopped meat broth (CMB) (Holdeman et al., 1977). Plates were incubated for 7 days at 37 °C. Inoculated plates were monitored daily for any change in color of the medium from the initial red to yellow (reaction of acid with phenol red). After 7 days, plates were removed from anaerobic incubation and the pH of the medium contained in each well was measured. A pH reduction of 1.0 as compared to that of uninoculated control wells was considered as indicating fermentation of CD by a given strain.

Replicator and Growth Medium. The defined medium of Varel and Bryant (1974), as adapted by Salyers et al. (1977), was used for replicator screening of CD-degrading organisms and for inducing enzymes. The basal medium contained the following: ammonium sulfate, 1.0 g/L; vitamin B-12, 5 μg/L; hemin, 5.0 mg/L; K_2HPO_4, 2.26 g/L; KH_2PO_4, 0.9 g/L; $FeSO_4{\cdot}7H_2O$, 4.0 mg/L; NaCl, 0.9 g/L; $CaCl_2{\cdot}2H_2O$, 0.027 g/L; $MgCl_2{\cdot}6H_2O$, 0.02 g/L; $MnCl_2{\cdot}4H_2O$, 0.01 g/L; $CoCl_2{\cdot}6H_2O$, 0.01 g/L; cysteine hydrochloride, 0.5 g/L; carbohydrates (CD), 5.0 g/L; phenol red, 0.1 g/L; agar, 15 g/L. All chemicals were of reagent grade. All components of the medium except cysteine hydrochloride and autoclaved CD were mixed, the pH was adjusted to 7.6, and the medium was autoclaved (15 min at 121 °C). Filter-sterilized cysteine hydrochloride and autoclaved CD solution were added to give a final substrate concentration of 0.5%. HPLC analysis showed no breakdown of CD occurred during autoclaving. The pH of the medium was readjusted to 7.6, and then the solution was dispensed into sterile microtiter plates (Wilkins and Walker, 1975). The plates were covered, cooled, and incubated aerobically at 37 °C for 24 h to check for contamination. Plates were placed in an anaerobic glove box (Coy Manufacturing Co., Ann Arbor, MI) for 4 days prior to inoculation to reduce O_2 tension. The pH of the medium dropped to about 7.1 during this equilibration, due to interaction with the CO_2 in the glovebox atmosphere.

The medium for growth and inducing enzymes was the same as the replicator medium except that no agar or phenol red was added, and all components except cysteine hydrochloride were mixed before boiling. The defined medium was prepared anaerobically according to the procedure of Holdeman et al. (1977). The medium was adjusted to pH 7.1 with NaOH, anaerobically dispensed into glass anaerobic culture tubes (18 × 1.5 cm, Bellco Glass Inc., Vineland, NJ), and stoppered under oxygen-free nitrogen. The tubes were placed in a tube press (Bellco Glass Inc., Vineland, NJ) and autoclaved. Tubes were incubated at 37 °C for 24 h to check for contamination.

Bacterial Growth. Two bacterial strains were selected from the replicator study for detailed examination. Both strains were transferred at 1% inoculum level into CMB and incubated at 37 °C for 18 h. Cultures were then diluted 1:100 in prereduced 1% peptone blanks for preenrichment, and 1% inoculum was transferred into CMB and incubated at 37 °C for 18 h. CD growth medium was then inoculated at the 5% level and incubated at 37 °C for 18 h. Inoculation was done under oxygen-free nitrogen using a VPI anaerobe culture system (Bellco Glass Inc., Vineland, NJ).

Preparation of Crude Cyclodextrinase. Test tubes containing 9.5 mL of the CD medium were inoculated with 0.5 mL of selected culture. The tubes were incubated at 37 °C. At 2-h intervals up to 18 h, tubes were removed from incubation for analysis. Bacterial growth was analyzed by measuring the absorbance (turbidity) of cultures at 600 nm with a Bausch & Lomb spectrophotometer (Rochester, NY). Bacterial cells were harvested to obtain extracellular crude enzyme fraction (ECF) and intracellular crude enzyme fraction (ICF) according to the general procedures developed by Dekker and Palmer (1981), outlined in Figure 1. The protein content of the ICF was determined by the method of Lowry et al. (1951).

Enzyme Assay. α- and β-CDs were used as substrates for assaying intra- and extracellular cyclodextrinase (CDase) activity. CD solutions at 10 mg/mL were prepared in phosphate buffer (0.05 M, pH 6.8). Tubes containing the CD solution were stoppered, placed into a tube press, and autoclaved. Assay mixtures were prepared by adding 1 volume of CD substrate solution to 1 volume of ECF or ICF. The final CD concentration was 5 mg/mL. The enzyme–substrate mixtures were incubated for 4 h at 37 °C and then boiled for 15 min to inactivate enzymes. A boiled enzyme control was tested in parallel with each set of assays. Upon cooling, the sample mixtures were centrifuged (17000*g*, 10 min, 4 °C). The supernatant fluid was analyzed for reducing sugar by the method of

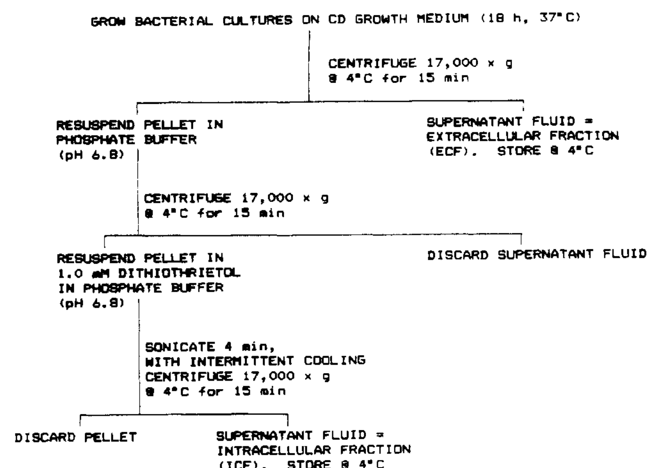

Figure 1. Scheme for the preparation of microbial enzyme fractions.

McFeeters (1979). Activity in this standard assay was expressed in terms of μmol/mL of reducing ends released as glucose in 4 h, under the conditions of the assay.

HPLC Analysis of Products of CD Hydrolysis. Two volumes of reaction mixture were mixed with 3 volumes of acetonitrile, to precipitate any material that would be insoluble in the eluent (70% acetonitrile) employed for HPLC analysis. The mixtures were then deionized by adding an equivalent volume of mixed bed ion-exchange resin (AG 501-X8, 100–200 mesh, Bio-Rad Laboratories, Richmond, CA) and stirring for 10 min at room temperature. Deionized samples were filtered through 0.3-μm glass fiber filters (Gelman, Ann Arbor, MI).

A μBondapak–carbohydrate column (Waters Associates, Milford, MA) was used for separating monosaccharides and short-chain oligosaccharides (Palmer, 1975, 1979; Conrad and Palmer, 1976). Samples were eluted with 70–30 or 80–20 (v/v) mixtures of acetonitrile and water, at a flow rate of 2.0 mL/min. Eluting mono- and maltooligosaccharides were detected with a refractive index detector and identified from their retention times as compared to standards.

Maltooligosaccharide standards of degree of polymerization (DP) ranging from 1 to 7 were prepared by acid hydrolysis of β-CD according to the method of Kondo et al. (1981).

Determining the Properties of Crude Cyclodextrinase. The temperature optimum profile was determined by incubating CDase with an equivalent volume of CD substrate solution (final CD concentration 5 mg/mL, pH 6.8) for 4 h at 4, 25, 37, 42, 55, and 80 °C. Mixtures were then analyzed for reducing sugar (as glucose) as previously described. The temperature stability was examined by incubating CDase at 4, 25, 37, 42, 55, and 80 °C for 48 h, followed by measurement of CDase activity (at 37 °C) by the standard assay. The pH optimum was determined by incubating CDase in an equivalent volume of buffered CD solution (final CD concentration 5 mg/mL) of pH 3, 4, 5, and 6 (citrate, 0.05 M), 7, 8, and 9 (Tris, 0.05 M), and 10 (carbonate, 0.05 M) for 4 h at 37 °C. The reducing sugar released (as glucose) was assayed as previously described. The pH stability was determined by incubating CDase in appropriate buffers of pH 3, 4, 5, and 6 (citrate, 0.05 M), 7, 8, and 9 (tris, 0.05 M), and 10 (carbonate, 0.05 M) for 48 h at 37 °C followed by measurement of CDase activity (at pH 6.8) by the standard assay.

RESULTS

Replicator Screening for CD Fermentation. The screening procedure showed that 24 of the 30 *Bacteroides* strains tested were able to degrade and grow on CDs (Table I). Although the final determination was made 7 days after inoculation by measuring the drop in pH, most of the 24 strains showed evidence (indicator color change) of growth at 24 h, as shown in Table I.

Table I. Results of Replicator Screening of Fermentation of CDs by Different DNA Homology Groups of *Bacteroides*

species	strain[a]	pH[b]
3452-A[c]	C10-2	6.52 ± 0.07
	C7-8	6.50 ± 0.10
	C14-3	6.61 ± 0.15
B. disatasonis	B1-20*[d]	5.16 ± 0.10
	C18-7*	4.69 ± 0.06
	C21-1*	5.10 ± 0.18
B. ovatus	R3-39*	5.12 ± 0.09
	0038-1*	5.53 ± 0.10
	T4-7	6.27 ± 0.06
	C1-45*	5.20 ± 0.09
	B4-11*	5.52 ± 0.14
	3524*	4.97 ± 0.06
B. eggerthii	B8-51	6.40 ± 0.02
B. thetaiotaomicron	7330-1*	5.77 ± 0.28
	B1-46*	5.23 ± 0.03
	5951*	5.27 ± 0.08
	T1-37	5.93 ± 0.05
	6180-A*	5.54 ± 0.10
B. uniformis	C7-17*	5.45 ± 0.15
	C31-12*	5.36 ± 0.11
	C20-25	6.25 ± 0.08
B. vulgatus	C43-46B*	4.94 ± 0.06
	C11-25*	5.55 ± 0.31
	OC-13	5.65 ± 0.12
	R1-5*	5.42 ± 0.07
	C10-6*	5.30 ± 0.05
B. fragilis type I	0479	5.73 ± 0.11
B. fragilis type II	A3-18B*	5.37 ± 0.18
	C40-1	5.26 ± 0.21
	C48-32*	5.21 ± 0.12

[a] Strains obtained from the VPI Anaerobe Laboratory culture collection. [b] Values represent the mean and the standard deviation ($n = 3$). A pH reduction below 5.80 was considered as indicating fermentation of CD by a given strain. [c] Unnamed DNA homology groups are designated by the number of the reference strain. [d] Asterisk indicates evidence of growth (indicator color change) at 24 h.

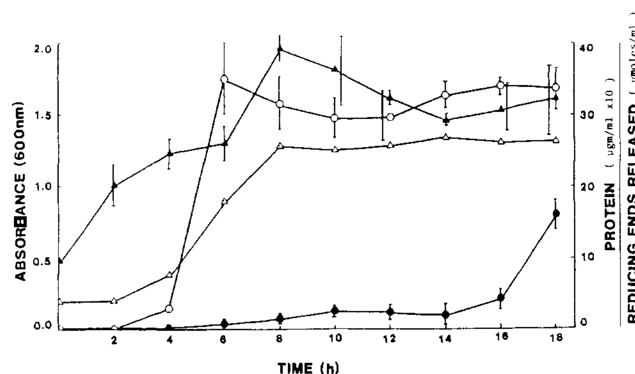

Figure 2. Protein synthesis and CDase activity during 18 h (37 °C) growth of *B. ovatus* 3524 on CD medium. Symbols: (△) growth measured by turbidity (600 nm); (▲) intracellular protein measured by the method of Lowry et al. (1951); (○) intracellular CDase activity; (●) extracellular CDase activity measured by the standard assay.

Production and Localization of CDase during Bacterial Growth. These studies were designed to correlate bacterial growth and protein production with the production and localization of CDase. Figure 2 shows the results for *Bacteroides ovatus* 3524. This organism entered the logarithmic growth phase after 2-h incubation and entered the stationary phase at 10 h. CDase activity was

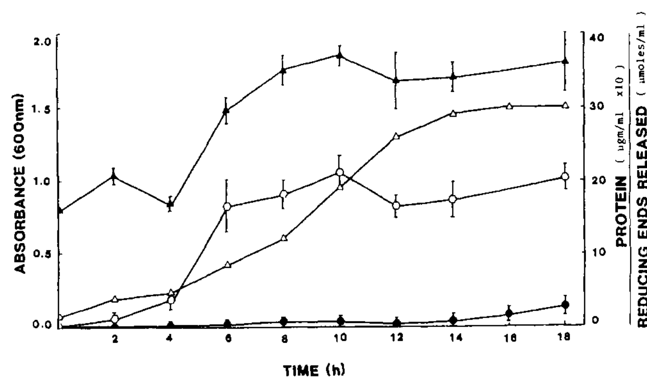

Figure 3. Protein synthesis and CDase activity during 18 h (37 °C) growth of *B. distasonis* C18-7 on CD medium. Symbols: (△) growth measured by turbidity (600 nm); (▲) intracellular protein measured by the method of Lowry et al. (1951); (○) intracellular CDase activity; (●) extracellular CDase activity measured by the standard assay.

primarily intracellular up to about 16-h growth, with a sharp increase in extracellular activity occurring between 16 and 18 h. Intracellular CDase activity for this strain was detected at 4-h growth, reaching maximum activity by 6 h.

Figure 3 shows similar data for *Bacteroides distasonis* C18-7. CDase activity in the ECF of *B. distasonis* C18-7 was especially low, with intracellular levels of CDase activity being about 6 times greater than extracellular levels after 18-h growth. CDase from this strain was detected at 2 h of growth, with maximum activity occurring at 10 h.

No CDase activity was detected in either strain when cells were grown on medium containing glucose as the carbon source. Thus, the CDase activity appears to be induced in both strains by growth on CDs.

Products of CD Hydrolysis. Elution with a 70–30 (v/v) mixture of acetonitrile and water proved suitable for routine rapid separation of α- and β-CDs from each other and for separation of the expected products of hydrolysis. However, maltotetraose (DP 4) was not separated from α-CD, nor was maltopentaose (DP 5) separated from β-CD. Where necessary, these pairs were separated in a more prolonged analysis by using 80–20 (v/v) acetonitrile–water as the eluent.

The products of CD hydrolysis were distinctly different for the two *Bacteroides* strains. After 18-h incubation, HPLC analysis showed that CDase from *B. distasonis* C18-7 catalyzed the breakdown of α-CD to glucose, maltose, and maltotriose. β-CD was hydrolyzed similarly to yield a mixture of glucose, maltose, maltotriose, and maltotetraose after 18-h incubation. In contrast, the CDase from *B. ovatus* 3524 hydrolyzed α- and β-CD completely to glucose in 18 h.

Time-course analysis via HPLC of CD hydrolysis by CDase from both strains was conducted to examine product formation during early stages of CD hydrolysis. At 1-, 3-, and 8-h incubation (37 °C), *B. ovatus* 3524 CDase again catalyzed only the production of glucose, with no significant presence of intermediate short-chain oligosaccharides (parts a, b, and c of Figure 4). In contrast, *B. distasonis* C18-7 CDase catalyzed formation of glucose–oligosaccharide mixtures at all stages, with the relative proportions of the individual products remaining approximately the same throughout (Figure 5). This indicates that the crude CDases from these two strains contain different enzymes.

The hydrolysis mixtures in Figures 4 and 5 were analyzed via the routine HPLC procedures. However, they

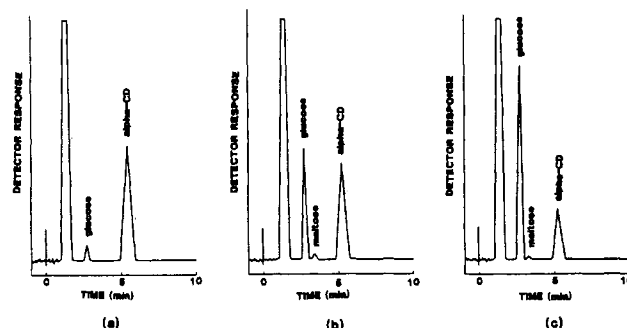

Figure 4. Time-course analysis via HPLC of α-CD hydrolysis by *B. ovatus* 3524 CDase at (a) 1, (b) 3, and (c) 8 h. Eluent (70% acetonitrile–30% water) at a flow rate of 2.0 mL/min, on a μ Bondapak–carbohydrate column; refractive index detector.

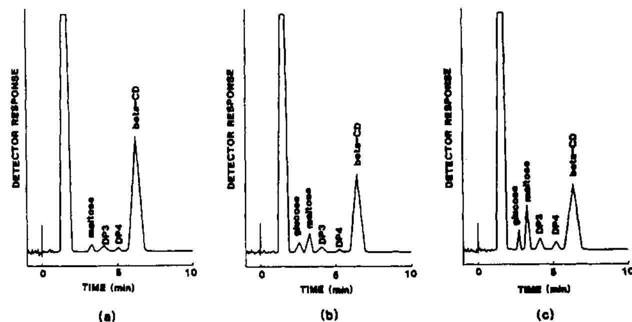

Figure 5. Time-course analysis via HPLC of β-CD hydrolysis by *B. distasonis* C18-7 CDase at (a) 1, (b) 3, and (c) 8 h. Conditions as in Figure 4.

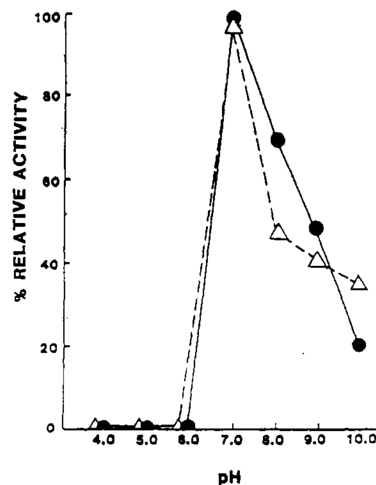

Figure 6. pH optimum (●) and stability (△) for *B. ovatus* 3524 CDase. pH optimum was assayed by measuring the increase in reducing ends (as glucose) after 4-h (37 °C) incubation of CDase on substrate solution. pH stability was measured by holding the enzyme at the designated pH for 48 h, after which CDase activity was measured by the standard assay.

were also analyzed by elution with 80–20 (v/v) acetonitrile/water and found to be free of maltooligomers larger than DP4.

Effect of Temperature and pH on Crude Cyclodextrinase Activity. The enzymes from both strains showed optimum pH activity and stability at neutral pH (Figures 6 and 7). However, *B. distasonis* C18-7 showed optimum pH activity and stability over a broader pH range (Figure 7). Further investigation of the properties of the isolated enzymes showed a temperature optimum of 55 °C for *B. distasonis* C18-7 (4-h incubation), with little enzyme activity lost when the enzyme was incubated at 4–55 °C for 48 h (Figure 8). *B. ovatus* 3524 showed a temperature optimum of 42 °C (4-h incubation), but there was a sig-

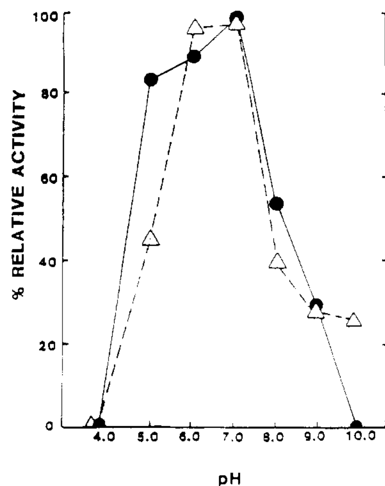

Figure 7. pH optimum (●) and stability (△) for *B. distasonis* C18-7 CDase. Conditions as in Figure 6.

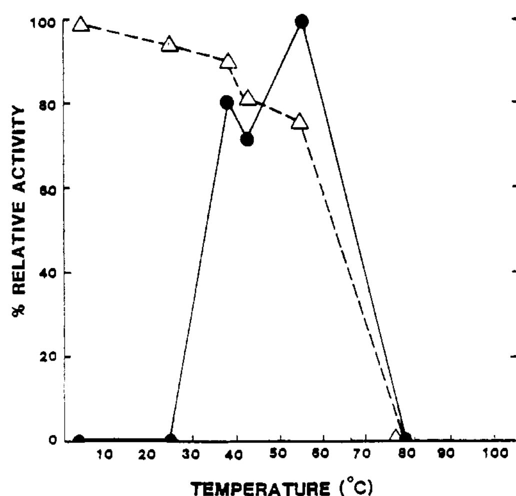

Figure 8. Temperature optimum (●) and stability (△) for *B. distasonis* C18-7 CDase. Temperature optimum was assayed by measuring the increase in reducing ends (as glucose) after 4-h (pH 6.8) incubation of CDase on substrate solution. Temperature stability was measured by holding the enzyme at the designated temperature for 48 h, after which CDase activity was measured by the standard assay.

nificant loss of activity when the enzyme was incubated at 37 °C and higher for longer than 24 h (Figure 9). However, despite this loss, complete hydrolysis of CDs to glucose by *B. ovatus* 3524 CDase occurs within 18 h at 37 °C in the assay system employed. Thus, it would appear that the enzymes from both strains would be stable and active in the colon environment.

CONCLUSIONS

CD use in food products raises concern about the metabolic fate of CD and about its possible toxicity to humans. Earlier evidence shows that CDs are metabolized in the rat and suggests the colon as a possible location of CD metabolism in the rat (Anderson et al., 1963; Szabo et al., 1981). The results obtained in the present study suggest that CDs will be extensively hydrolyzed in the human colon. Most (24 of 30) of the selected *Bacteroides* strains were able to degrade CDs as evidenced by their ability to grow on CDs as the sole carbon source. More detailed investigation of CDase isolated from two selected *Bacteroides* strains showed that CDase activity was predominantly cell bound and induced by as little as 2-4-h growth on CDs. The enzymes were shown to be stable and active under pH and temperature conditions (pH 6.80; 37 °C)

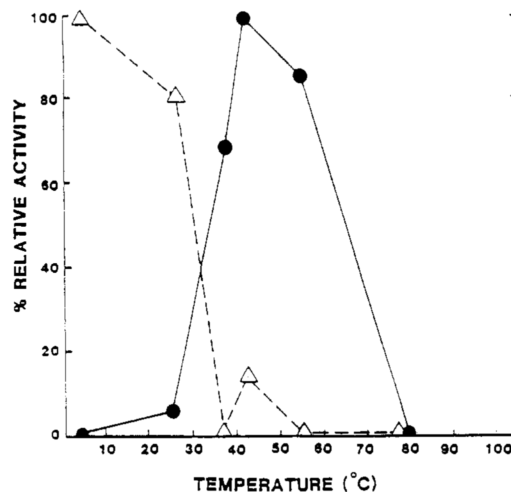

Figure 9. Temperature optimum (●) and stability (△) for *B. ovatus* 3524 CDase. Conditions as in Figure 8.

expected in the colon environment. Under these conditions, the CDases catalyzed extensive degradation of CDs in 18 h, and degradation was well under way in as little as 1–2 h.

Taken together, these results suggest that a typical 40-h transit time through the colon (Burkitt and Painter, 1975) would be more than adequate time to permit induction of CDase and subsequent partial or complete hydrolysis of CDs by the induced enzymes. These results are consistent with earlier studies showing that *Bacteroides* can degrade a variety of food carbohydrates via enzymes induced by growth on the carbohydrates (Salyers et al., 1977; Dekker and Palmer, 1981; Vercellotti et al., 1977). This ability to induce enzymes suggests that members of the *Bacteroides* genus are able to adapt to available substrates in order to compete for survival in the human colon environment (Salyers et al., 1977).

The products of CD hydrolysis include glucose and maltooligosaccharides, already known to be readily fermentable by colon anaerobes to yield fatty acids and flatus gases, among other products. Thus, ingestion of CDs could lead to nutritional and/or physiological effects.

The CD hydrolysis mixtures were also found to be free of maltooligomers larger than DP4. Therefore, CD-hydrolyzing enzymes from both strains appear to exhibit a high degree of "multiple attack", in which an enzyme attacks a single substrate molecule several times before attacking another substrate molecule (Robyt and French, 1964). Since all of the studies on product formation were done with crude enzyme preparations, it is also possible that the products formed resulted from the continued attack of more than one enzyme. Isolation of purified individual enzymes will be required to further elucidate the actual mechanism of hydrolysis.

Pure bacterial cultures grown on CD as the sole carbon source were used in this research to examine breakdown of CDs in vitro by human colon anaerobes. The use of mixed bacterial cultures and/or providing the organisms with a choice of growth substrates would better approximate the colon environment. However, limited information about the actual colon environment makes simulation difficult. Future determination of possible in vivo factors of CD hydrolysis will involve examining the ability of these organisms to synthesize CDases in the presence of other substrates and organisms.

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Registry No. α-CD, 10016-20-3; β-CD, 7585-39-9; CDase, 37288-41-8.

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Diterpenoid Sweeteners. Synthesis and Sensory Evaluation of Biologically Stable Analogues of Stevioside

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Two analogues, 2′,3′,19-tri-*O*-(sodiosulfopropyl)steviolmonoside (**6**) and 2″,3″,3′,19-tetra-*O*-(sodiosulfopropyl)steviolbioside (**7**), of the biologically labile diterpenoid triglycoside Stevioside (**1**) were prepared. Under conditions simulating those of the human GI tract, **6** and **7** were found to be completely stable while **1** was converted completely to the aglycon, steviol. The purpose of this work was to obtain sweet analogues of **1** possessing a safety advantage over **1** due to minimal GI-tract absorption resulting from increased molecular weight and charge. Neither **6** nor **7** exhibited significant sweet taste quality. The sensory properties of **1**, nine other analogues of **1**, and six other nonnutritive sweeteners, including saccharin, Acesulfam-K, sodium cyclamate, Aspartame, neohesperidin dihydrochalcone, and monoammonium glycyrrhizzinate, are also presented.

The public interest in development of safe nonnutritive substitutes for carbohydrate sweeteners is very high. This has been demonstrated most conclusively by the strong consumer acceptance of Aspartame, a dipeptide sweetener composed of the two natural amino acids, L-Phe and L-Asp. In this case, safety for human consumption was anticipated from the natural nutrient composition. Our approach toward the development of a safe nonnutritive sweetener has been entirely different. Our strategy assumes that a sweet compound that is nonabsorbable through the gastrointestinal (GI) tract will be devoid of toxicity. Earlier, we reported results of a study on the effect of charge and molecular weight on GI-tract absorption of some dihydrochalcone sweeteners (Wingard et al., 1978). In essence, a cutoff for significant (≤1%) absorption was found to occur at approximately 1000 daltons. In addition, increased negative charge was observed to reduce absorption. This result would be anticipated if one assumes a phospholipid membrane model for GI-tract cells. Here anionic species would experience charge repulsion during approach to the membrane.

Recently, we reported that the 19-*O*-β-D-glucosyl moiety of Stevioside (**1**) (Scheme I) could be replaced by a sodiosulfopropyl moiety to give **2**, a compound where the sweet taste character was not only retained but also improved considerably (DuBois et al., 1981b). As a bonus, it found that, whereas **1** is rapidly metabolized to the potentially toxic (Vignais et al., 1966) aglycon, steviol (**3**), **2** gives only the dessophoro derivative **4**. Although it was found that the 13-*O*-β-D-sophorosyl moiety of **2** could not be substituted by other polar groups (e.g., sodiosulfopropyl) without loss of sweet taste character, it has been deter-

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